

WHAT IS CLAIMED IS:

1. A method of transiently permeabilizing one or more cells, comprising:
  - a) maintaining said one or more cells in a substantially stationary position within an effective distance from a solid surface; and
  - b) directing to said solid surface electromagnetic radiation sufficient to induce transient permeabilization of a membrane of said one or more cells, without prior knowledge of the specific three-dimensional location of said one or more cells, wherein said one or more cells is coincident with the path of said electromagnetic radiation.
2. The method of Claim 1, wherein said electromagnetic radiation has an energy density at said solid surface selected from the group consisting of at most about 0.001, 0.002, 0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1, 2, 3, 4, 5 and 6  $\mu\text{J}/\mu\text{m}^2$ .
3. The method of Claim 1, wherein said electromagnetic radiation has an energy density at said solid surface of about 0.001 to about 0.3  $\mu\text{J}/\mu\text{m}^2$ .
4. The method of Claim 1, wherein said effective distance is selected from the group consisting of less than about 1000  $\mu\text{m}$ , 600  $\mu\text{m}$ , 300  $\mu\text{m}$ , 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , 60  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$ , 10  $\mu\text{m}$ , 6  $\mu\text{m}$ , 3  $\mu\text{m}$ , 2  $\mu\text{m}$ , and 1  $\mu\text{m}$ .
5. The method of Claim 1, wherein said effective distance is between about 1  $\mu\text{m}$  to about 20  $\mu\text{m}$ .
6. The method of Claim 1, wherein said electromagnetic radiation is directed to said one or more cells for a period of time selected from the group of at most on the order of 1000 seconds, 100 seconds, 10 seconds, 1 second, 100 milliseconds, 10 milliseconds, 1 millisecond, 100 microseconds, 10 microseconds, 1 microsecond, 100 nanoseconds, 10 nanoseconds, 1 nanosecond, 100 picoseconds, 10 picoseconds, 1 picosecond, 100 femtoseconds, 10 femtoseconds, 1 femtosecond, 100 attoseconds, 10 attoseconds, and 1 attosecond.

7. The method of Claim 1, wherein said one or more cells are exposed to said electromagnetic radiation for a period of time of about 100 picoseconds to about 10 seconds.

8. The method of Claim 1, wherein said directing comprises delivering a pulse of radiation to said solid surface.

9. The method of Claim 1, wherein said directing comprises passing a beam of radiation across said solid surface according to a path pattern.

10. The method of Claim 1, further comprising inducing transient permeabilization of a membrane in said one or more cells at a rate that is selected from the group of at least 10, 30, 100, 300, 1000, 3000, 10,000, 30,000, 100,000, 300,000, 1,000,000, 3,000,000, 10,000,000, 30,000,000, 100,000,000 and 240,000,000 cells per second.

11. The method of Claim 1, further comprising inducing transient permeabilization in a membrane of said one or more cells at a rate of between about 300 to about 10,000,000 cells per second.

12. The method of Claim 1, wherein the probability of viability of said one or more cells after said transient permeabilizing of a membrane of said one or more cells is maintained at a value selected from the group consisting of at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% and 99%.

13. The method of Claim 1, wherein the probability of viability of said one or more cells after said transient permeabilizing is maintained at a value of at least 50% to at least 90%.

14. The method of Claim 1, further comprising contacting said one or more cells with a non-isotonic aqueous medium.

15. The method of Claim 1, further wherein said one or more cells contacts an aqueous medium that lacks a substance, or contains the substance at a concentration lower than the concentration of the substance within said one or more cells, such that said substance within said one or more cells can exit said one or more cells through a transiently permeabilized membrane.

16. The method of Claim 15, wherein said substance is selected from the group consisting of an ion, an organic molecule, an inorganic molecule, a colloidal particle, a polysaccharide, a peptide, a protein, a nucleic acid, and a modified nucleic acid.

17. The method of Claim 1, further wherein said one or more cells contacts an aqueous medium such that a substance within said aqueous medium can enter said one or more cells through a transiently permeabilized membrane.

18. The method of Claim 17, wherein said substance is selected from the group consisting of an ion, an organic molecule, an inorganic molecule, a colloidal particle, a polysaccharide, a peptide, a protein, a nucleic acid, and a modified nucleic acid.

19. The method of Claim 17, further wherein said transiently permeabilized membrane recovers to a substantially non-permeabilized state within a period of time selected from the group consisting of at most about 0.3 millisecond, 1 millisecond, 3 milliseconds, 10 milliseconds, 30 milliseconds, 100 milliseconds, 300 milliseconds, 1 second, 3 seconds, 10 seconds, 30 seconds, 1 minute, 2 minutes, 3 minutes, 6 minutes, 10 minutes, 20 minutes and 30 minutes.

20. The method of Claim 17, wherein said transiently permeabilized membrane recovers to a substantially non-permeabilized state within a period of time of about 1 second to about 1 minute.

21. The method of Claim 1, wherein said electromagnetic radiation is directed to an area of said solid surface at a rate that is selected from the group consisting of at least

about 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 200, 300 and 400 square centimeters per second.

22. The method of Claim 1, wherein said electromagnetic radiation is directed to an area of said solid surface at a rate of about 0.0003 to about 10 square centimeters per second.

23. The method of Claim 1, wherein said directing comprises delivering two or more pulses of radiation to said solid surface at a rate selected from the group of at least 1, 10, 100,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  Hz.

24. The method of Claim 1, wherein said directing comprises delivering two or more pulses of radiation to said solid surface at a rate of about  $10^2$  to about  $10^4$  Hz.

25. The method of Claim 1, wherein said electromagnetic radiation originates from an energy source selected from the group consisting of a continuous wave laser, a pulsed laser, a continuous lamp, and a flashlamp.

26. The method of Claim 1, wherein said directing comprises delivering two or more pulses of electromagnetic radiation to said solid surface according to a pulse target pattern.

27. The method of Claim 26, wherein an individual pulse of said pulses of electromagnetic radiation has a duration selected from the group consisting of at most on the order of 1000 seconds, 100 seconds, 10 seconds, 1 second, 100 milliseconds, 10 milliseconds, 1 millisecond, 100 microseconds, 10 microseconds, 1 microsecond, 100 nanoseconds, 10 nanoseconds, 1 nanosecond, 100 picoseconds, 10 picoseconds, 1 picosecond, 100 femtoseconds, 10 femtoseconds, 1 femtosecond, 100 attoseconds, 10 attoseconds, and 1 attosecond.

28. The method of Claim 26, wherein an individual pulse of said pulses of electromagnetic radiation has a duration from about 100 picoseconds to about 10 seconds.

29. The method of Claim 26, wherein at least two pulses of electromagnetic radiation are directed to a single pulse target within said pulse target pattern.

30. The method of Claim 1, wherein said electromagnetic radiation is directed to a defined area on said solid surface, and said defined area has an area selected from the group consisting of at least 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 200, 300 and 400 square centimeters.

31. The method of Claim 1, wherein said electromagnetic radiation is directed to a defined area on said solid surface, and said defined area has an area of about 0.0001 to about 10 square centimeters.

32. The method of Claim 1, wherein said electromagnetic radiation is directed simultaneously to substantially the entirety of said defined area.

33. The method of Claim 1, wherein said path of said electromagnetic radiation has a width selected from the group of at least 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 300,  $1 \times 10^3$ ,  $2 \times 10^3$ ,  $3 \times 10^3$ ,  $4 \times 10^3$ ,  $5 \times 10^3$ ,  $6 \times 10^3$ ,  $7 \times 10^3$ ,  $8 \times 10^3$ ,  $9 \times 10^3$  and  $1 \times 10^4$  micrometers.

34. The method of Claim 1, wherein said path of said electromagnetic radiation has a width of about 10 micrometers to about 1000 micrometers.

35. An apparatus for transiently permeabilizing a cell, comprising:

a) an energy source that emits electromagnetic radiation sufficient to induce transient permeabilization of a membrane of a cell, wherein said cell is substantially stationary and contained within a defined volume, wherein the specific coordinates of said cell within said defined volume are unknown, and wherein said defined volume is partly bounded by a solid surface;

b) a directing device configured to direct said electromagnetic radiation to substantially the entirety of said defined volume, wherein said cell is coincident

with the path of said electromagnetic radiation, and wherein said electromagnetic radiation within said defined volume has an energy density at said solid surface of at most about  $6 \mu\text{J}/\mu\text{m}^2$ ; and

c) said solid surface.

36. The apparatus of Claim 35, wherein said electromagnetic radiation within said defined volume has an energy density at said solid surface selected from the group consisting of at most about 0.001, 0.002, 0.003, 0.006, 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1, 2, 3, 4 and  $5 \mu\text{J}/\mu\text{m}^2$ .

37. The apparatus of Claim 35, wherein said electromagnetic radiation within said defined volume has an energy density at said solid surface of about 0.001 to about 0.3  $\mu\text{J}/\mu\text{m}^2$ .

38. The apparatus of Claim 35, wherein said directing device directs pulses of electromagnetic radiation to said defined volume according to a pulse target pattern.

39. The apparatus of Claim 37, wherein an individual pulse of said pulses of electromagnetic radiation has a duration selected from the group consisting of at most on the order of 1000 seconds, 100 seconds, 10 seconds, 1 second, 100 milliseconds, 10 milliseconds, 1 millisecond, 100 microseconds, 10 microseconds, 1 microsecond, 100 nanoseconds, 10 nanoseconds, 1 nanosecond, 100 picoseconds, 10 picoseconds, 1 picosecond, 100 femtoseconds, 10 femtoseconds, 1 femtosecond, 100 attoseconds, 10 attoseconds, and 1 attosecond.

40. The apparatus of Claim 37, wherein an individual pulse of said pulses of electromagnetic radiation has a duration of about 10 seconds to about 100 picoseconds.

41. The apparatus of Claim 37, wherein at least two pulses of electromagnetic radiation are directed to a single pulse target within said pulse target pattern.

42. The apparatus of Claim 35, wherein said path of said electromagnetic radiation has a width selected from the group consisting of at least 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 300,  $1 \times 10^3$ ,  $2 \times 10^3$ ,  $3 \times 10^3$ ,  $4 \times 10^3$ ,  $5 \times 10^3$ ,  $6 \times 10^3$ ,  $7 \times 10^3$ ,  $8 \times 10^3$ ,  $9 \times 10^3$  and  $1 \times 10^4$  micrometers.

43. The apparatus of Claim 35, wherein said path of said electromagnetic radiation has a width of about 10 micrometers to about 1000 micrometers.

44. A system with a memory comprising a set of instructions, such that when executed the computer performs the action comprising directing to a solid surface electromagnetic radiation sufficient to induce transient permeabilization of a membrane of a substantially stationary cell, without prior knowledge of the specific three-dimensional location of said cell, wherein said cell is coincident with the path of said electromagnetic radiation.